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OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

SUBJECT: **EPA R7 ESE Alcohol, Inc. Final Report - ACB Project B21-14**

Date: **July 20, 2022**

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And

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INTRODUCTION

The Analytical Chemistry Branch (ACB), Office of Pesticide Programs, U.S. Environmental Protection Agency (EPA) was requested by the EPA Region 7 to screen for multiple pesticides at the residue level in five (5) wastewater samples and five (5) sludge/soil samples, along with two (2) water field blanks and one (1) soil field blank samples. This memorandum includes the table of analytical results (Attachment 1), and the first, second, and glyphosate extraction methods (Attachment 2a, b, and c).

SAMPLE DELIVERY, RECEIPT, AND INSPECTION

Five (5) soil samples, five (5) water samples, two (2) water field blanks, and one (1) soil field blank were received under chain of custody on March 18, 2021. A list of the samples can be found in Table 1. All samples were received in good condition with original seal intact. The samples were immediately transferred to the refrigerator upon receipt at the ACB laboratory and kept cold until time of analysis.

Table 1. List of samples received at ACB.

Project ID	Sample ID	Sample Description	Receiving Date
JAHESE	01	Soil/Sludge	3/18/2021
	02	Soil/Sludge	
	03	Soil/Sludge	
	04	Soil/Sludge	
	05	Soil/Sludge	
	001	Liquid, with H_2SO_4 as preservative	
	002	Liquid, with H_2SO_4 as preservative	
	003	Liquid, with H_2SO_4 as preservative	
	004	Liquid, with H_2SO_4 as preservative	
	005	Liquid, with H_2SO_4 as preservative	
	FB1	Liquid blank, with H_2SO_4 as preservative	
	FB2	Soil blank	
	FB3	Liquid blank, with H_2SO_4 as preservative	

SAMPLE PROCESSING AND ANALYSIS

The methods used in this analysis are attached (see Attachment 2). Briefly, the samples were processed by extracting about 10 g of sample aliquots with organic solution and cleaned up using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method. The sample extracts were analyzed using high performance liquid chromatography/tandem mass spectrometry (LC/MSMS) in positive electrospray mode. Two MS/MS transitions were monitored for each compound. The transition that produced the greatest response and had the least interference was used as the quantitation signal. The other transition was used as confirmation. Table 2 lists the requested target analytes and requested quantitation limits for each analyte.

Because of the diverse nature of the target analytes, some of the analytes required a separate extraction, cleanup, and analysis method. Some requested analytes produced low-quality signals or required extractions or analysis that were beyond the capabilities of ACB. These analytes are marked in the table below and in Attachment 1.

Table 2. Requested target analytes and LOQs

Analytes	LOQ	Analytes	LOQ
Abamectin	3	Ipconazole	96
Acetamiprid		Isavuconazole	
Azoxystrobin	1,200	Itraconazole[1]	
Bifenthrin		Mancozeb[1]	53
Brassinazole		Mefenoxam/Metalaxyl	474
Captan	830	Metconazole	300
Carbendazim		Nitenpyram	
Carboxin		Orysastrobin	
Chlorantraniliprole	10,100	Penflufen	2,400
Chlorpyrifos	1.8	Permethrin (1-2)	
Chlorpyrifos-methyl		Picoxystrobin	290
Clothianidin	630	Posaconazole	
Cyantraniliprole	60	Propiconazole	600
Cyfluthrin (1-4)		Prothioconazole	60
Cyhalothrin (1-2)		Pyraclostrobin	
Cypermethrin (1-4)		Ravuconazole	
Cyproconazole		Sedaxane	690
Deltamethrin (1-2)		Sulfonic acid prothioconazole[1]	
Desthio-prothioconazole		Tebuconazole	190
Difenoconazole	60	Tetraconazole	
Dimoxystrobin		Thiabendazole	210
Dinotefuron		Thiacloprid	
Epoxiconazole		Thiamethoxam	77
Ethaboxam	350	Thiophanate methyl	
Fluconizole		Thiram[1]	96
Fludioxonil	200	Tolclofos methyl	
Fluoxastrobin	96	Trifloxystrobin	240
Glufosinate[2]		Uniconazole	
Glyphosate[2]		Voriconazole	
Imidacloprid	360		

[1]=These compounds were not analyzed due to instrument/method limitations. Itraconazole is only soluble in DCM and is not compatible with LC/MS analysis. Due to its high MW and polarity, analysis on GC/MS is not successful. Mancozeb is a polymeric compound and not compatible with the instrument used in this study. Prothioconazole sulfonic acid degrades quickly during analysis. No reliable response can be acquired. Thiram degrades quickly in both water and soil matrices, and during analysis. No reliable response can be acquired.

[2]=Glyphosate and glufosinate were partially analyzed. See explanation in *Results and Discussion.*

Note: Actual LOQs for compounds generally much lower than requested values. Generally, 1-10 ppb.

The samples were extracted and analyzed twice, and the results were labeled and reported as preliminary (the first round of extraction and analyses) and the final results (the second round of extraction and analyses), respectively. For the first round of extraction and analyses, the water samples were extracted within 14 days of receipt and the sludge/soil samples within 21 days. Preliminary results were compiled and reported to R7. As elaborated below in *Results and Discussion*, poor spike recovery was observed for many of the compounds. After further improvement and fine tuning of the methods

for extract cleanup and sample analyses, and the verifications of the performance of the methods, all the samples were reextracted and reanalyzed. Results from this re-extraction and re-analysis were reported as the final results.

Quality Assurance Measurements

In addition to the general quality control measurements described in ACB's policies and SOPs, a procedural blank, a fortified laboratory control blank sample, and a fortified field blank sample were processed concurrently for each batch of samples. Field blank samples of water or sludge/soil were processed along with the field samples of water or sludge/soil. All the standards were purchased from a commercial vendor with certificate of analysis indicating the concentration or purity of the standard. Some standards were obtained from the EPA's National Pesticide Standard Repository. Instrument calibration standards were run with each batch of samples for the quantitation. Results were reported as ppb (nanogram/g) (wet weight) of the samples.

RESULTS AND DISCUSSION

The results from both the first and the second extractions are provided in Attachment 1. The results between the first and the second extraction are generally in agreement. Ten analytes were found at very high levels in both the water and soil samples: Azoxystrobin, Chlorantraniliprole, Clothianidin, Ethaboxam, Fludioxonil, Ipconazole, Metalaxyl, Tebuconazole, Thiabendazole, and Thiamethoxam. Many other analytes were detected at low levels in both water and soil samples.

The calibration range of the analytical instrument was about 1 ppb to 300 ppb equivalency of sample concentrations (5 ng/ml to 1000 ng/ml of standard solutions) in the method. For analytes with concentrations exceeding the maximum calibration limit, the sample extracts were diluted by various degrees to bring the responses and concentrations of the analytes within the calibration range for quantitation. For the ten analytes (listed above) with very high concentrations, the sample extracts have to be diluted by up to 10,000 times in order to bring the analyte responses on the instrument within the calibration range. Because of this large magnitude of change in the matrix amount in the diluted extracts and the propagation and magnifying of the initial uncertainty in the sample processing method by dilution, the concentration values calculated from diluted sample extracts have higher uncertainty level and are, therefore, treated as estimated.

One notable difference between the first and the second analyses is clothianidin concentration in sludge/soil sample #05. The second analysis reports a value an order of magnitude higher than that from the first analysis. Upon further examination of the analytical raw data, it was found that the poor calibration quality of clothianidin in the dilutions of the first extraction batch has caused a much lower value for clothianidin. A carry-over of clothianidin on the instrument from the analysis of undiluted sample previously has distorted the instrument calibration curve, which resulted in the lower calculated clothianidin concentration in this diluted sample. If these affected calibration standards were not used and if only the initial calibration standards were used, the re-calculated clothianidin concentration in this sample from the first extraction would be closer (230,000 ppb) to the value from the second extraction.

There were some differences in the calculated values of many analytes between the first and second extractions. Most of the differences are as expected from the inherent variations in the sample processing and analyses. Dilutions would further increase the possible variations in the results. In

addition, some changes in the sample cleanup method and in the analytical parameters, although for improvement, likely introduced further variations in the results between the first and the second extractions and analyses.

For the first extraction, all sample extracts were analyzed on a C18 HPLC column, the details of which can be found in Attachment 2. The surprisingly high concentrations of the ten analytes in the sample extracts overloaded the analytical instrument. Despite of the extensive cleaning of the analytical instrument, the residual amount of these ten analytes were still present in the analytical system and could not be completely removed. Carry-over and high baselines were observed even after multiple blank injections which prevented accurate quantitation of diluted samples due to the effects on calibrations standard. Because the responses of the target compounds in the diluted samples were orders of magnitude greater than the high baseline caused by the carry-over, ACB proceeded with the analyses. After optimization of the methods, including replacing the analytical column and optimizing the analytical parameters, samples were re-extracted and re-analyzed. The re-analyses on the instrument started with diluted extract first to avoid overloading the instrument. No carry-over was observed with the second extraction and analyses. Instrument calibrations were robust. As a results of the further optimization of the cleanup methods and the instrument parameters, and an improved approach to reduce the carry-over of the high concentration analytes on the LC/MSMS, the results from the second extractions are considered to be more accurate.

During the sample analysis, low levels of contamination with some target analytes were detected in the field blank samples. Coincidentally, the same analytes observed at high levels in the field samples were detected in soil blank FB2 and water blank FB1 at low levels (below 300 ppb). Because the laboratory procedural blanks and field blank FB3 that were processed along with all the samples are free of contamination in both of the first and the second extractions, it appears that the cross-contamination of the FB1 and FB2 with the high concentration analytes likely had happened in the field. However, the possibility of the cross-contamination happened in the laboratory cannot be ruled out. Because the levels of the ten analytes detected in the field blanks (FB1 and FB2) are low, as compared to the concentrations found in the field samples, the samples and sample results should be valid and not adversely affected.

The % recoveries of some compounds in the method verification fortifications from FB2 were very high. These compounds with high recoveries were also found in the FB2, likely due to cross-contamination, which renders the % recovery values of these compounds invalid. The estimated amount of the cross-contamination of these six compounds is about 10 to 100 ppb. If the levels of these compounds were taking into consideration in the calculations, the fortification recoveries would fall within acceptable range.

Glyphosate and glufosinate required separate extraction and analysis methods from the other compounds listed in Table 2. At the time of the sample analyses, ACB had a working method for water and vegetation matrices. ACB proceeded with sample processing and analysis for both the water and sludge/soil samples using the existing water and vegetation methods. In short, about 10 grams of matrix are extracted with dilute phosphonic acid and cleaned up by filtration and SPE (See Attachment 2c). During the first round of extractions, both water and soil samples were processed for analysis with the glyphosate method. No glyphosate and glufosinate were detected. However, the method performance for the sludge/soil samples were poor, with low recoveries from fortified samples.

Additional modification and development of the method for sludge/soil did not improve the method performance much. Because these two compounds are hydrophilic (water soluble), and are mobile across soil matrices, they are likely not be present in the sludge/soil samples if no residue was found in the water samples associated with the sludge/soil. Due to time constraints, no additional efforts were made to develop a method for the analysis of these two compounds in sludge/soil matrices.

Finally, a correction was made for one analyte previously reported in the Preliminary (the first round of extraction) Data. Carbendazim was initially reported in the sludge/soil samples S#02, #03, #04, and #05 at 30, 130, 450, and 41 ppb, respectively. A calculation error was found relating to the conversion between instrument-calculated values and the residue levels in the samples. The attached Result Table reflects the correct values for the first round of extraction.

The analytical results and sample processing procedures have been subject to ACB internal quality assurance review. Findings and notations of the QA review are documented in the ACB QA review sheets.

Attachment 1. Result Table - Preliminary and final results of sample analyses

See Excel Spreadsheet included with electronic report.

Attachment 2. Methods Used for Sample Analyses

See PDF Files included with electronic report.